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(54) Title: TREATMENT AND PREVENTION OF DECUBITUS

(57) Abstract: The present invention provides a method for treating a subject suffering from or at risk of suffering from decubitus, the method comprising a step of administering erythropoietin (EPO), or a functional part, derivative or analogue thereof to said subject. In certain embodiments, the EPO has been recombinantly produced in host cells that further express the E1A protein of an adenovirus.

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Treatment and prevention of decubitus.

#### FIELD OF THE INVENTION

5 The present invention relates to the field of medicine. More in particular the invention relates to therapies that aim to treat and/or prevent decubitus.

#### BACKGROUND OF THE INVENTION

10 Decubitus (pressure ulcers, pressure sores, bed sores) develops in the skin and subcutaneous tissues that are subjected to prolonged pressure as occurs in areas that carry weight of the body in bed ridden patients. The clinical symptoms of pressure sores can be divided in four stages  
15 (Oxford Handbook of Clinical Specialities, 5th Edition, 1999): Stage I: non-blanching erythema over intact skin; Stage II: partial thickness skin loss, e.g. shallow crater; Stage III: full thickness skin loss, extending into fat; Stage IV: destruction of muscle, bone, or tendons. Preventive measures  
20 consist of the avoidance of pressure points anywhere on the body surface by use of air and water cushions, mattresses, regular change of position of the body and the limbs, and stimulation of the circulation in the areas at risk. Treatment of decubitus is often difficult and the lesions can be very  
25 painful. The prevention and treatment of decubitus poses a large burden on nursing. 7% of inpatients have pressure sores. Up to 85% of paraplegic patients have pressure sores. The emphasis is on prevention as treatment of existing ulcers is time consuming and cumbersome. Therefore a need exists for  
30 further therapies for the prevention and/or treatment of decubitus. The present invention aims at providing such therapies.

BRIEF DESCRIPTION OF THE FIGURES

- Fig. 1. Surface of ulcers developing after 8 hours of pressure in MF-1 mice (example 5.A). Values are means; bars represent standard deviations. t(d): time (days). Squares: buffer; triangles: EPO treated. 3.3  $\mu$ g EPO was administered intraperitoneally 1 hour before the application of and immediately after the release of pressure.
- Fig. 2. Surface of ulcers developing after 10 hours of pressure in Balb/c mice (example 5.B). Values are means; bars represent standard deviations. t(d): time (days). Squares: buffer; triangles: EPO treated. 4.5  $\mu$ g EPO was administered intraperitoneally 1 hour before the application of pressure.
- Fig. 3. Surface of ulcers developing after 6 hours of pressure in MF-1 mice (example 5.C). Values are means; bars represent standard deviations. t(d): time (days). Squares: buffer; triangles: EPO treated. 4.5  $\mu$ g EPO was administered intravenously 10 minutes before the application of pressure.

DESCRIPTION OF THE INVENTION

The invention provides a method for treating a subject suffering from or at risk of suffering from decubitus ulcers, the method comprising a step of administering erythropoietin, or a functional part, derivative or analogue thereof to said subject. The invention provides for use of erythropoietin or a functional part, derivative or analogue thereof for the treatment and/or prevention of decubitus ulcers. The invention provides a use of erythropoietin or a functional part, derivative or analogue thereof for the preparation of a medicament for treatment and/or prevention of decubitus

ulcers. In certain embodiments, the erythropoietin, functional fragment, derivative or analogue thereof according to the invention has been recombinantly produced in host cells that further express the E1A protein of an adenovirus, preferably  
5 PER.C6® cells.

In another aspect the invention provides a pharmaceutical preparation for the treatment or prevention of decubitus ulcers, characterized in that said preparation comprises erythropoietin, or a functional part, derivative or analogue  
10 thereof. In certain embodiments hereof, said pharmaceutical preparation is suitable for topical administration.

#### DETAILED DESCRIPTION OF THE INVENTION

For the present invention, the definition of decubitus is  
15 meant to include the development or risk of development of skin ulcers due to prolonged pressure on an area of the skin, and the terms 'pressure sores', 'pressure ulcers' and 'bed sores' are meant to be included into the definition of decubitus. The definition includes all stages of the  
20 conditions indicated by these terms, such as the four stages described in the background of the invention hereinabove, starting with non-blanching erythema over intact skin.

In the present invention, EPO is used for treatment of subjects suffering from or at risk of suffering from decubitus  
25 ulcers. In certain embodiments of the invention, the treatment of a subject with erythropoietin is aimed at avoiding effects of EPO believed to be undesired when treating or preventing conditions of decubitus ulcers. To that effect EPO variants, fragments, derivatives or analogs are used that have decreased  
30 erythropoietic effect as such or due to pharmacokinetic properties. In one preferred embodiment the EPO contains lewis-X structures on its N-linked glycans, preferably on

average at least about 1.5 lewis-X structures per EPO molecule. In accordance with one preferred method the EPO is a low sialylated version of EPO, preferably having on average less than 10, more preferably less than 8, still more preferably less than 6 sialic acid moieties per EPO molecule.

In accordance with another preferred embodiment such EPO variant is obtainable by recombinantly producing EPO in a cell that is characterized by low sialylation of recombinant proteins produced therein. Examples of such cells are adenovirus E1-expressing cells. In one preferred embodiment thereof, said cells are cells derived from PER.C6 cells, expressing E1 and EPO, or a fragment, derivative or analog thereof. In certain embodiments, the treated subjects are not anaemic. The present invention for the first time discloses the use of EPO for the treatment of decubitus irrespective of whether the hematocrit value (red blood cell count) of the patient is lower than normal or not. This provides decubitus per se as a novel indication for the use of EPO. The present invention therefore provides for the use of EPO for treatment of patients with decubitus, wherein said patients do not necessarily have another indication besides decubitus, which would otherwise have warranted the treatment of such a patient with EPO based on the presently available knowledge. A non-anaemic patient as used herein, is a patient that has a hemoglobin value that is considered as being within the normal range, which value would not otherwise lead a physician to prescribe EPO to this patient, i.e. absent knowledge of the current invention. Up till now, application of EPO is mostly restricted to the prevention or correction of anaemia in specific patient populations, including the (pre)dialysis phase of chronic renal insufficiency, cytostatic therapy, premature infants and as preparation for autologous blood

transfusion or surgical procedures with anticipated major blood loss. The general aim in such cases is to increase hemoglobin levels (Hb) by increasing the number of red blood cells (hematocrit) to a specific range by adapting standard  
5 dosage regimes to individual needs. Depending on the patient population, the optimal Hb level ranges from a lower limit of 6.5-7.5 mmol/L to an upper limit of 8.0-8.7 mmol/L. Hence, in certain embodiments of the present invention, the subjects treated with EPO for decubitus are not patients in the  
10 (pre)dialysis phase of chronic renal insufficiency, undergoing cytostatic therapy, premature infants, or patients undergoing autologous blood transfusion or surgical procedures with anticipated major blood loss.

EP patent application no. 1072609 relates to  
15 cytoprotective agents comprising prosaposin-related peptides, and describes that peptides referred to as 18-MP already at concentrations in the picomolar to femtomolar range promote expression of Bcl-X<sub>L</sub> protein, and suppress apoptosis-like neuron death. Based on these observations, a wide range of  
20 clinical applications for 18-MP is envisaged, including treatment of peripheral tissue diseases accompanied by apoptosis or apoptosis-like cell death. Bedsore is one of the conditions mentioned. Based on the finding that EPO enhances expression of Bcl-X<sub>L</sub> protein at similarly low doses as well, it  
25 is suggested that EPO has the same effect and efficacy as 18-MP. No proof of the action of 18-MP for treatment of decubitus is presented in EP 1072609. Furthermore, it should be noted that not all apoptosis involves the Bcl-X<sub>L</sub> pathway. In any case, the molecular reasons for the clinical symptoms of  
30 decubitus are complex and presently not completely understood, and the involvement of apoptosis in the clinical symptoms of decubitus is unclear, if existent. On the contrary, decubitus

is characterized at certain stages by necrosis (e.g. Witkowski JA and Parish LC (1982), J Am Acad Dermatol 6: 1014-1021). Necrosis is clearly distinct from apoptosis (e.g. Paus R et al (1993) Exp Dermatol 2: 3-11). In fact, apoptosis has been reported to appear concurrently with reepithelialization of the wound and may signal the end of the inflammatory phase of healing at that site in the wound (Brown et al (1997) Surgery 121: 372-380), and therefore induction of apoptosis might even be beneficial rather than causal to skin wounds. A link between apoptosis and decubitus therefore appears fully unclear. In addition a causal relationship between any agent inducing Bcl-X<sub>L</sub> and treatment and/or prevention of decubitus appears to include at least one extra level of uncertainty. The present invention for the first time provides the use of EPO for treatment and/or prevention of decubitus.

A subject according to the present invention may be an animal, and in preferred aspects is a human subject.

Erythropoietin (EPO) is a glycoprotein hormone, which in humans has a molecular weight of 34 to 38 kD. The glycosyl residues comprise about 40% of the molecule. The role of EPO that has been studied and put to practice most is in the production of red blood cells. Recently other uses have been envisaged for EPO, such as the protection, restoration and enhancement of EPO-responsive cells (WO 02/053580). It is the merit of the present invention to describe the novel use of EPO for the treatment or prevention of decubitus ulcers.

Many forms of EPO, as well as functional fragments, derivatives and analogues thereof have been described, and it will be clear that all these are included within the scope of the present invention for the prevention and/or treatment of decubitus ulcers.

EPO according to the invention is EPO as may be isolated from

any suitable source. Preferably, human EPO is recombinantly produced and isolated from a suitable recombinant host cell and/or from the culture medium. In the case of recombinant production, the host may suitably be chosen from any cell

5 capable of recombinantly producing protein, such as bacterial host cells (e.g., E.coli, B.subtilis), yeast (e.g., S.cerevisiae, K.lactis), fungi (e.g., A.niger, Pichia), mammalian cells (e.g., CHO, BHK) including human cells. According to one aspect of the invention, EPO is recombinantly

10 produced in a human cell line, in particular an immortalized human embryonic retina cell line expressing E1A of an adenovirus, such as a PER.C6 cell line. It is also possible to administer EPO in a gene-therapy setting according to the invention, for instance by treating a patient with a vector

15 comprising a nucleic acid sequence capable of expressing EPO when delivered to a target cell.

Derivatives of EPO refer to modifications of the source EPO, which may be urinary EPO, or EPO recombinantly producible from a cDNA or gene sequence, wherein the expression product has

20 one or more modifications relative to the source EPO, which modifications may be in the primary structure, by substitution of one or more amino acid residues (such as in Novel Erythropoiesis Stimulating Protein (NESP)), deletion, addition or relocation of one or more amino acid residues, or

25 alterations in the post- or peri-translational modification of the protein backbone, such as hydroxylations, phosphorylations or glycosylations of amino acid residues, sulphur bridges, and the like. Derivatives also encompass naturally or non-naturally occurring EPO variants coupled to non-EPO related

30 proteinaceous moieties or even to non-proteinaceous moieties. EPO and derivatives useful for the present invention include both native erythropoietins as well as erythropoietins that



have been altered by at least one modification as compared to native erythropoietin, and preferably as compared to native human erythropoietin. The at least one modification may be a modification of at least one amino acid of the erythropoietin molecule, or a modification of at least one carbohydrate of the erythropoietin molecule. Of course, erythropoietin molecules useful for the purposes herein may have a plurality of modifications compared to the native molecule, such as multiple modifications of the amino acid portion of the molecule, multiple modifications of the carbohydrate portion of the molecule, or at least one modification of the amino acid portion of the molecule and at least one modification of the carbohydrate portion of the molecule. Derivatives of EPO are encompassed by the instant invention, as long as they interact with the EPO receptor and cause a reduction or prevention of decubitus. This can be tested by methods known to the person skilled in the art, such as those according to the examples provided herein.

Functional analogues of EPO refer to molecules not necessarily derived from naturally or non-naturally occurring EPO, that are capable of mimicking the interaction of EPO with its receptor, whereby decubitus is reduced and/or prevented. Such functional analogues may comprise peptidomimetics and/or non-peptidic molecules mimicking the idiotope interacting with the EPO-R. It will be understood by those of skill in the art, that the functional analogue according to the invention need not necessarily interact with the same idiotope, or in the same way, as long as its interaction mimics the interaction of EPO with its receptor. Functional analogues may suitably be screened and selected from (synthetic) peptide libraries, phage or ribosome polypeptide display libraries, or small molecule libraries. Those of skill in the art are capable of

screening for, or designing functional analogues, and test their functionality in assays disclosed herein.

The forms of EPO useful in the practice of the present invention encompass naturally occurring, synthetic and

5 recombinant forms of erythropoietin, including but not limited to urinary EPO, brain-EPO, renal EPO, serum-EPO, etc. By way of non-limiting example, forms of EPO useful for the practice of the present invention include EPO muteins, such as those with altered amino acids at the carboxy terminus described in

10 US patents 5,457,089 and 4,835,260; agonists described in US patent 5,767,078; EPO isoforms with various numbers of sialic residues per molecule, such as described in US patent 5,856,292; polypeptides described in US patent 4,703,008; peptides which bind to the EPO receptor as described in US

15 patents 5,773,569 and 5,830,851; small-molecule mimetics which activate the EPO receptor, as described in US patent 5,835,382; and EPO analogues as described in WO 95/05465, WO 97/18318 and WO 98/18926. All these citations are incorporated herein to the extent that such disclosures refer to the

20 various alternate forms or processes for preparing such forms of EPO of the present invention. EPO can be obtained commercially (for instance under the trademarks of PROCRIT, available from Ortho Biotech; EPOGEN, available from Amgen, Inc; EPREX, available from Jansen-Cilag).

25 Glycosylation of the EPO molecule can have impact on its functionality. Any glycosylation form of EPO is encompassed in the present invention. EPO that is now commercially available and used for increasing the red blood cell counts (hematocrit) of patients in need thereof can be used according to the

30 invention. In certain embodiments however, the EPO used for the present invention has less erythropoietic activity. In one embodiment, the EPO of the invention has at least no sialic

acid moieties. In another embodiment, the modified EPO is asialoerythropoietin. In another embodiment, the modified EPO has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 sialic acid moieties. In another embodiment, the modified EPO has at least  
5 no N-linked or no O-linked carbohydrates. In another embodiment, the modified EPO has at least a reduced carbohydrate content by virtue of treatment of EPO with its native carbohydrates with at least one glycosidase, or by virtue of having a mutation in at least one of the amino acids  
10 to which an N-linked or O-linked carbohydrate is bound in natural EPO. In another embodiment, the modified EPO has less N-linked tetra-antennary carbohydrate chains than natural EPO.

It will be immediately clear that an EPO molecule according to the invention can conveniently be produced in  
15 recombinant host cells, wherein genetic information encoding EPO is present in expressible format. Molecular biology provides convenient methods well known to the person skilled in the art, for generating the genetic information that encodes any kind of EPO, fragments, derivatives or analogues  
20 thereof. The expression of recombinant nucleic acid molecules in host cells is also well known in the art. This provides the advantage of an easy to manipulate, renewable source of EPO with the same characteristics, in contrast to purification of EPO from natural sources, which may be more burdensome. It  
25 will therefore immediately be clear that wherever a functional fragment or derivative is described by deleting and/or mutating and/or adding amino acids from/to EPO, this can be conveniently brought about by deletions and/or mutations and/or additions to the EPO coding sequence that is used for  
30 recombinant expression. Hence, in certain embodiments, the EPO, or functional fragment, derivative or analogue thereof is recombinantly expressed in a host cell. In certain embodiments

thereof, said host cell is a cell that expresses the E1A protein of an adenovirus. Preferably said cell is derived from a human retina cell. Most preferably said cell is derived from a PER.C6 cell. Recombinant production of EPO in PER.C6 cells  
5 has been described in WO 00/63403, which is incorporated by reference herein to the extent that it describes methods for the production of EPO in PER.C6 cells. EPO that is produced in host cells that express the E1A protein of an adenovirus has a specific glycosylation pattern, described in WO 03/038100,  
10 herewith incorporated by reference in its entirety. This EPO has less erythropoietic activity than commercially available EPREX. A low erythropoietic activity may be an advantage for the treatment of decubitus patients with low mobility, as an increase in hematocrit for such patients could be seen as an  
15 undesired side-effect of EPO. In certain preferred aspects of the invention therefore, used EPO molecules comprise on average at least 0.5 Lewis-X structures (which may include sialyl-Lewis-X structures) per N-linked glycan (i.e. at least 1.5 Lewis-X structures per EPO molecule). Preferably in these  
20 aspects, the EPO molecules on average comprise less than 10 sialic acid moieties, more preferably less than 8, still more preferably less than 6 sialic acid moieties per EPO molecule.

EPO according to the present invention can be of any source, but preferably is human EPO when human subjects are to  
25 be treated. The use of human EPO may prevent an immunogenic response against the administered protein. Of course, human EPO encompasses EPO protein that has been obtained from human tissue or fluid, such as blood or urine, as well as EPO that has been recombinantly produced in host cells, wherein the  
30 nucleic acid sequence encoding said EPO is a sequence encoding human EPO. Preferably said host cells are human cells. In certain embodiments of the invention, EPO is administered

in a gene-therapy setting, i.e. nucleic acid comprising the coding sequence of EPO is administered, e.g. in a viral vector, such as an adenovirus vector which includes the EPO coding sequence in expressible format. Administration of the nucleic acid encoding EPO, e.g. transdermally, intradermally, or systemically, and the like, to a subject suffering from or at risk of suffering from decubitus, will result in EPO being expressed and having its therapeutic and/or prophylactic effect in the treated subject. The person skilled in the art knows how to prepare gene delivery vehicles, such as recombinant adenovirus, comprising nucleic acid sequences encoding EPO, a derivative, fragment or analogue thereof, and administer these to a subject, e.g. by local injection. It will therefore be clear to the skilled person that a gene-therapy setting wherein nucleic acid comprising the coding sequence of EPO in expressible format is administered to a subject is included within the meaning of 'administering EPO' according to the present invention.

According to the present invention EPO can be administered to patients at risk of developing decubitus ulcers, prior to the manifestation of symptoms, to preclude the development of ulcers. Alternatively, or additionally, according to the invention EPO may be administered to patients soon after the manifestation of clinical symptoms, such as redness and painfulness of the affected body parts. Further according to the invention EPO may be administered to patients that already suffer from decubitus ulcers, to improve their condition. In preferred embodiments the EPO is administered in the early stages of the process, such as stage I characterized by the non-blanching erythema over intact skin. According to another preferred embodiment, a patient at high risk for developing pressure sores is treated prophylactically, i.e.

before clinical symptoms are observed, with EPO on a regular basis.

According to the invention, EPO may be administered systemically, for instance intravenously, or by local  
5 injection, for instance subcutaneously, or topically, or in any other form known to the person skilled in the art.

Treatment regimes for erythropoietic purposes are well established. In general EPO dosages are given in IU (international units), referring to the activity of EPO in  
10 erythropoiesis. Such IU correlate to the protein content of EPO but are operationally defined, and hence the correlation may vary between different batches. As a rule of thumb, one IU corresponds to 8-10 ng epoetin alfa. It will be clear to the person skilled in the art that although the IU are usually  
15 given for commercial EPO preparations, the concentration of EPO molecules in such preparations can easily be defined according to standard procedures. This will allow to determine the relative specific activity e.g in IU/g (see e.g. EP 0428267). In certain embodiments, the administered dose of EPO  
20 or EPO equivalent according to the present invention is in the range of 0.1-1000000 IU per kg body weight, preferably in the range of 1-10000 IU per kg body weight, when administered intravenously or subcutaneously. The dose for instance depends on the route of administration. This may of course also depend  
25 on the form of EPO used, e.g. on the erythropoietic potential of the used EPO. Normal doses of EPO that are administered to adult renal failure patients are in the range of 4000 - 7500 IU per week (80 - 100 kg body weight). These amounts are normally divided into 3 separate doses per week for the  
30 commercially available epoetin alpha or Eprex (EPO produced on CHO cells). For the present invention such doses are suitable. In other embodiments, higher doses may be given daily or even

more frequently. The maximum tolerable dose may have to be determined, in order to prevent hematocrit values and hemoglobin concentrations to rise too sharply. Persons of ordinary skill know how to monitor hematocrit values and hemoglobin concentrations in patients to prevent undesired side effects, such as extreme high blood pressure that may occur in later stages of the treatment. For improved results, it may be required to administer EPO for a longer period. For this purpose doses may range from 10 to 100000 IU per administration, preferably 1000 to 2500 IU per administration (for an adult of 80 - 100 kg). Also in this case monitoring may be necessary to prevent unwanted side effects. For less erythropoietic EPO, such as EPO that has been recombinantly produced in PER.C6 cells, higher doses may be administered, e.g. 5000 IU/kg.

EPO according to the invention may be in the form of a pharmaceutically acceptable composition, as known to the person skilled in the art. The compositions may comprise *inter alia* stabilising molecules, such as albumin or polyethylene glycol, or salts. Preferably, the salts used are salts that retain the desired biological activity of EPO and do not impart any undesired toxicological effects. Examples of such salts include, but are not limited to, acid addition salts and base addition salts. Acid addition salts include, but are not limited to, those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include, but are not limited to, those derived from alkaline earth metals, such as sodium,

potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like. If necessary, EPO may  
5 be coated in or on a material to protect it from the action of acids or other natural or non-natural conditions that may inactivate the EPO. Furthermore, the present invention pertains to pharmaceutical compositions comprising at least EPO, a functional fragment, derivative or analogue according to the  
10 invention, The pharmaceutical composition of the invention further comprises at least one pharmaceutically acceptable excipient. A pharmaceutical composition according to the invention can further comprise at least one other therapeutic and/or prophylactic agent. These may include an anti-  
15 inflammatory agent, such as hydrocortisone, prednisone and the like. They may also include analgesic agents such as salicylic acid, acetaminophen, ibuprofen, flurbiprofen, morphine and the like. Such agents are all well known to the skilled person. Preferably, said further therapeutic and/or prophylactic  
20 agents are agents capable of preventing and/or treating decubitus ulcers. Typically, pharmaceutical compositions are sterile and stable under the conditions of manufacture and storage. The EPO can be in powder form for reconstitution in the appropriate pharmaceutically acceptable excipient before  
25 or at the time of delivery. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously  
30 sterile-filtered solution thereof. Alternatively, the EPO can be in solution and the appropriate pharmaceutically acceptable excipient can be added and/or mixed before or at the time of



delivery to provide a unit dosage injectable form. Preferably, the pharmaceutically acceptable excipient used in the present invention is suitable to high drug concentration, can maintain proper fluidity and, if necessary, can delay absorption.

5       The choice of the optimal route of administration of the pharmaceutical compositions will be influenced by several factors including the physico-chemical properties of the active molecules within the compositions, the urgency of the clinical situation and the relationship of the plasma  
10 concentrations of the active molecules to the desired therapeutic effect. For instance, if necessary, the EPO of the invention can be prepared with carriers that will protect it against rapid release, such as a controlled release formulation, including implants, transdermal patches, and  
15 microencapsulated delivery systems. Biodegradable, biocompatible polymers can *inter alia* be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Furthermore, it may be necessary to coat the EPO with, or co-administer the  
20 EPO with, a material or compound that prevents the inactivation of the EPO. For example, the EPO may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent.

      The routes of administration of the EPO according to the  
25 present invention include, but are not limited to, bolus, oral, buccal, epidermal, inhalation, intra-arterial, intradermal, intramuscular, intraperitoneal, intrasternal, intravenous, subcutaneous, topical, etc. Preferred administration routes according to the invention include  
30 intravenous injection or infusion or local injection at the site of the lesion. In another preferred embodiment, EPO is administered topically to the skin.

The pharmaceutical compositions of the present invention can also be formulated for parenteral administration. Formulations for parenteral administration can be *inter alia* in the form of aqueous or non-aqueous isotonic sterile non-toxic injection or  
5 infusion solutions or suspensions. The solutions or suspensions may comprise agents that are non-toxic to recipients at the dosages and concentrations employed such as 1,3-butanediol, Ringer's solution, Hank's solution, isotonic sodium chloride solution, oils such as synthetic mono- or  
10 diglycerides or fatty acids such as oleic acid, local anaesthetic agents, preservatives, buffers, viscosity or solubility increasing agents, water-soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like, oil-soluble  
15 antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like, and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric  
20 acid, and the like.

For topical administration, the preparations may be in the form of a lotion, gel, emulsion, ointment, bioadhesive composition, and the like. Besides EPO, or a functional fragment, derivative or analogue thereof, these preparations  
25 may for instance comprise one or more of aloe vera, tocopherol acetate, glycerine, stearic acid, 1-hexadecanol, polysorbate 60, apricot kernel oil, glyceryl stearate, PEG-100 stearate, dimethicone, PCP, allantoin, triethanolamine, carboner-940, etc., the general additions known to the skilled person.  
30 Further they may comprise hyaluronic acid and/or a cromolyn compound (US patent 6,573,249), which are beneficial for

treating decubitus ulcers. They may also include anti-inflammatory agents, analgesics, and the like.

The molecules are typically formulated in the compositions and pharmaceutical compositions of the invention  
5 in a therapeutically or diagnostically effective amount. Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). Furthermore, for example, a single bolus may be administered, several divided doses may be administered over time or the dose may be  
10 proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The molecules and compositions according to the present invention are preferably sterile. Methods to render these molecules and compositions sterile are well known in the art. The exact dosing regimen is  
15 usually sorted out during clinical trials in human patients.

Next to that, kits comprising at least EPO, or a functional fragment, derivative or analogue thereof, for the treatment of decubitus are also a part of the present invention. Optionally, the components of the kits of the  
20 invention are packed in suitable containers and labeled for prophylaxis and/or treatment of the indicated conditions. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile,  
25 solution or as a lyophilized, preferably sterile, formulation for reconstitution. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable  
30 by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's

- solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes. Associated with the kits can be instructions customarily
- 5 included in commercial packages of therapeutic or prophylactic products, that contain information about for example the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic or prophylactic products.
- 10 For the present invention, certain embodiments of a pharmaceutical composition comprise agents used in formulations for topical administration, as described above.

- The invention will now be illustrated with the following
- 15 examples, which are not to be construed to limit the scope of the invention.

#### EXAMPLES

- 20 In the following examples, the administration of two forms of EPO to the subjects (n=10 per group) is compared to each other, and to a negative control. The negative control is buffer (placebo), the two forms of EPO are commercially available EPO, such as EPREX, and EPO that has been produced
- 25 on PER.C6 cells (WO 00/63403, WO 03/038100). The dosage used is 100-5000 IU/kg. The EPO is administered intravenously or locally, i.e. subcutaneously at the site of the pressure. EPO is administered just before, during or preferably directly after the release of every episode of the pressure.

30

#### EXAMPLE 1

Model of chronic pressure ulcer in the rat.

In this model chronic pressure ulcers are induced by a device consisting of a subcutaneously implanted ferromagnetic steel plate and an externally applied magnet as described in (Pierce SM et al. (2000), Wound Rep Reg 8: 68-76).

- 5 EPO or placebo is administered systemically or locally after completion of each pressure application. The damage in the skin and subcutaneous tissues is quantitatively estimated at fixed time points after the termination of the pressure applications. The parameters recorded are blood flow in the
- 10 pressurized area, the size of the necrotic area that develops and histological determinants of inflammation and necrosis in the skin and underlying tissues. It is expected that the injury measured in the groups receiving EPO will be less than in the groups receiving placebo treatment.

15

#### EXAMPLE 2

##### **A different model of chronic pressure ulcer in hairless rats.**

- In this model pressure is applied on the skin area over the trochanter major of the rat. The surface pressure is computer
- 20 controlled according to the methods described in (Salcido R et al. (1995) J Rehab Res Develop 32: 149-161). The administrations of EPO and placebo as well as the measured endpoints are similar to those described in example 1. It is expected that in the animals treated with EPO the degree of
- 25 injury will be reduced as compared to the animals given placebo.

#### EXAMPLE 3

##### **Pressure induced skin injury in pigs.**

- 30 In this model pressure is applied on discrete areas of the dorsal skin of pigs over the femoral trochanters and damage is measured histologically at various times after the start of

reperfusion according to methods described in (Houwing R et al. (2000) J Wound Care 9: 36-44). In this case the test compounds will be administered systemically or locally before or after the completion of pressure application. It is  
5 expected that tissue injury will be less following treatment with EPO as compared to treatment with placebo.

#### EXAMPLE 4

##### **New model for decubitus: pressure induced ulcers in mice.**

10 In this model pressure is applied to the dorsal skin and subcutaneous tissues of mice by positioning two magnetic discs, one on each opposing side of a skin fold for a certain time period. The magnetic discs are held in place by the magnetic force which is equivalent to a pressure on the  
15 skinfold of approximately 450 kPa. The surface of the discs is circular with a diameter of 8 mm. The mice can be of the hairless type such as mice of the strain MF-1 and KH, or mice with a normal pelt. In the latter case the skin is shaved with an electrical pair of clippers prior to the application of the  
20 magnetic discs.

The magnetic pressure device is held in place for a predetermined period of time by the magnetic force on the unanaesthetized animals.

After the magnets have been removed, the tissue that was  
25 pressurized between the magnets appears pale and bloodless. By intravenous injection of a vital dye into these mice, it was demonstrated that the circulation in the previously compressed tissue has been disrupted. It also appears from the same dye technique that reperfusion of these tissues is slowly re-  
30 established within about 30 minutes. These observations demonstrate that the induction of pressure ulcers by this method is due to ischemia/reperfusion, similar to the

mechanism assumed to prevail in the causation of clinical bed sores.

The next few days, the skin in the compressed areas on the back of the mouse, one at each side of the dorsal midline, is  
5 yellowish and erythematous and develops crusts. By day 3 or 4 after release of the pressure, these areas become dark brown and take the appearance of ulcers.

Histological examination of these wounds confirmed the presence of epidermolysis, necrosis and inflammation of the  
10 underlying tissues, which penetrates into the muscle layer of the panniculus. In all aspects these wounds resemble those of severe pressure ulcers, but bone is obviously absent from the deeper layers of the lesion.

Between day 4 and 7 the surface of the ulcers may become  
15 moisty from wound exudate, and crust formation continues. The surface of the ulcers was measured with vernier callipers and daily measurements revealed that the ulcer area begins to decrease at day 4 to 5. Depending on the duration of exposure to pressure the ulcers have completely healed between 10 and  
20 16 days after release of pressure in the sense that complete re-epithelialization of the skin has occurred, and that the deeper layers have been replaced by scar tissue.

#### EXAMPLE 5

##### 25 **Treatment with EPO decreases the size of pressure ulcers**

Using the quantification of the surface area of the lesions as described in example 4, it was shown in 3 different experiments that treatment with EPO decreases the size of the resulting ulcers.

30 The EPO that was used in the following experiments, was produced in PER.C6 cells (as deposited at the ECACC under no. 96022940), and has a specific glycosylation pattern (WO

03/038100). In particular, the EPO used for these experiments is characterized by the presence of Lewis-X (and/or sialyl-Lewis-X, which are included in the definition of Lewis-X) structures on the N-linked glycans, and in particular the  
5 presently used EPO contained around 0.6 Lewis-X structure per N-linked glycan (EPO contains 3 N-linked glycans). It contains about 5 sialic acids per molecule (for comparison: the commercially available EPREX contains about 12.1 sialic acids per molecule, when measured using the same analysis method  
10 (based on quantification of an iso-electric focussing gel)). For comparison, the EPO as produced in PER.C6 cells and used for these experiments appears at least about 25 times less active in red blood cell production than commercially available EPREX.

15

A. In the first experiment, hairless MF-1 female mice were exposed to the magnetic pressure device described in example 4 for 8 hours. A group of 4 mice was treated twice with 3.3 µg of EPO. Treatment was by intraperitoneal injection of EPO in a  
20 volume of 0.5 ml at 1 hour before the application of pressure and immediately after the release of the pressure. A control group of 6 mice was given similar treatment with the solvent only.

The results are depicted in Fig. 1. It shows that the curve  
25 for the EPO treated mice runs below that of the controls, suggesting smaller lesions upon EPO treatment, but the difference is not statistically significant ( $p=0.18$ ) when the data are subjected to analysis of repeated measures.

30 B. The second experiment was performed with Balb/c female mice, which have a normal pelt. The shaved dorsal skinfold was subjected to pressure for 10 hours. One group of 8 mice was



treated with 4.5 µg of EPO. Treatment was by intraperitoneal injection of EPO in a volume of 0.5 ml at 1 hour before the application of pressure. A control group of 8 mice was given similar treatment with the solvent only.

5 The results are depicted in Fig. 2. Again, the curve for the EPO treated mice runs below that of the controls. Using analysis of repeated measures the difference in size between the EPO treated and the control mice is statistically significant ( $p=0.04$ ).

10

C. In a third experiment, hairless MF-1 mice were used and the treatment was performed by intravenous injection of EPO given 10 minutes before the application of the magnetic device. The pressure was maintained for a period of 6 hours. One group of 15 3 females and 3 males was injected with 4.5 µg of EPO using an injection volume of 0.5 ml. The control group consisting of 3 females and 4 males received similar treatment with the solvent only.

The results are depicted in Fig. 3. As in the previous 20 experiments, the average surface area of the ulcers of the EPO treated mice is smaller than that of the controls. The difference closely approaches statistical significance ( $p=0.06$ ) according to analysis of repeated measures.

25 When all data of the 3 experiments described above are subjected together to an analysis of repeated measures the ulcers of the EPO treated mice are on the average 3.3 mm<sup>2</sup> smaller and this difference is highly significant ( $p=0.01$ ).

30 These findings indicate that EPO treatment provides protection against pressure ulcer formation in that the resulting ulcers are smaller than without treatment. The graphs also show that

as a consequence of the smaller ulcer size, the time required for healing of the ulcers, i.e. to achieve complete re-epithelialization is reduced by 2-3 days.

CLAIMS

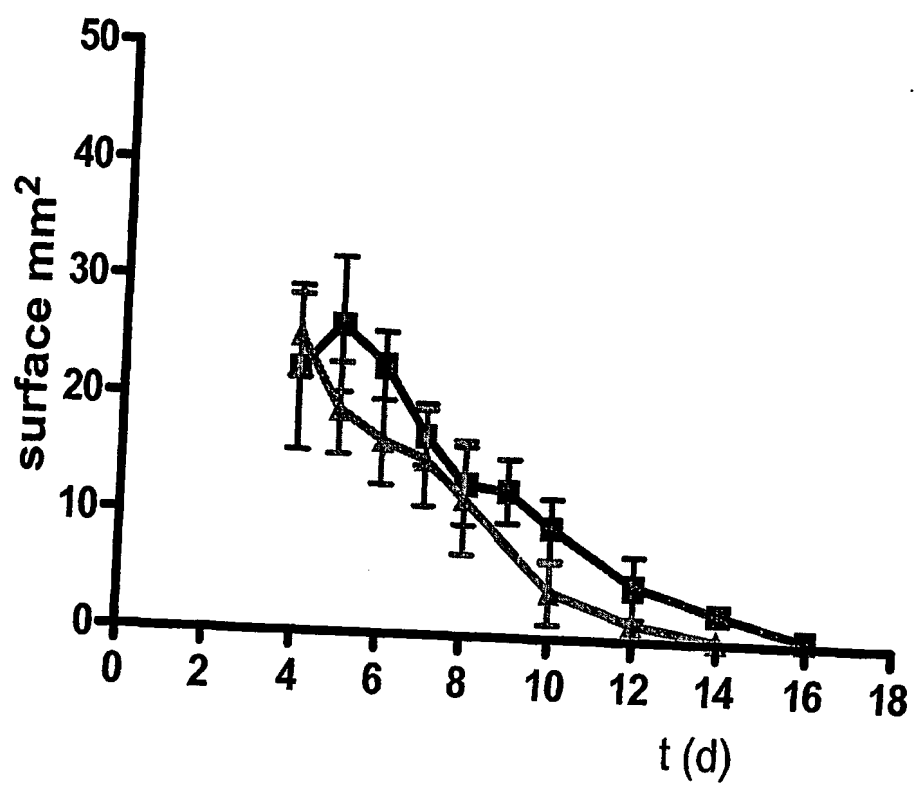
1. A method for treating a subject suffering from or at risk  
of suffering from decubitus, the method comprising a step  
5 of administering erythropoietin, or a functional part,  
derivative or analogue thereof to said subject.
2. A method according to claim 1, wherein said subject is  
not anaemic.
- 10 3. A method according to claim 1 or claim 2, wherein said  
subject is a human subject.
4. A method according to any one of claims 1-3, wherein said  
15 administering is performed during stage I of the clinical  
symptoms.
5. A method according to any one of claims 1-3, wherein said  
administering is performed before clinical symptoms are  
20 observed.
6. Use of erythropoietin or a functional part, derivative or  
analogue thereof for the treatment and/or prevention of  
decubitus.
- 25 7. Use of erythropoietin or a functional part, derivative or  
analogue thereof for the preparation of a medicament for  
treatment and/or prevention of decubitus.
- 30 8. A pharmaceutical preparation for the treatment of  
decubitus ulcers, characterized in that said preparation  
comprises

- a) erythropoietin, or a functional part, derivative or analogue thereof, and
- b) an analgesic and/or an anti-inflammatory agent.

- 5      9. A method according to any one of claims 1-5, use  
according to claim 6 or claim 7, or a pharmaceutical  
preparation according to claim 8, wherein said  
erythropoietin has been recombinantly produced in host  
cells that further express the E1A protein of an  
10      adenovirus.
10. A method, use, or pharmaceutical preparation according  
to claim 9, wherein said host cells are derived from  
primary human retina cells.
- 15      11. A method, use, or pharmaceutical preparation according  
to claim 10, wherein said host cells are derived from  
PER.C6 cells.
- 20      12. A method according to any one of claims 1-5, use  
according to claim 6 or claim 7, or a pharmaceutical  
preparation according to claim 8, wherein said  
erythropoietin comprises on average at least 1.5 Lewis-X  
structure per erythropoietin molecule.
- 25      13. A method, use, or pharmaceutical preparation according  
to claim 12, wherein said erythropoietin comprises on  
average less than 10 sialic acid moieties per  
erythropoietin molecule.
- 30

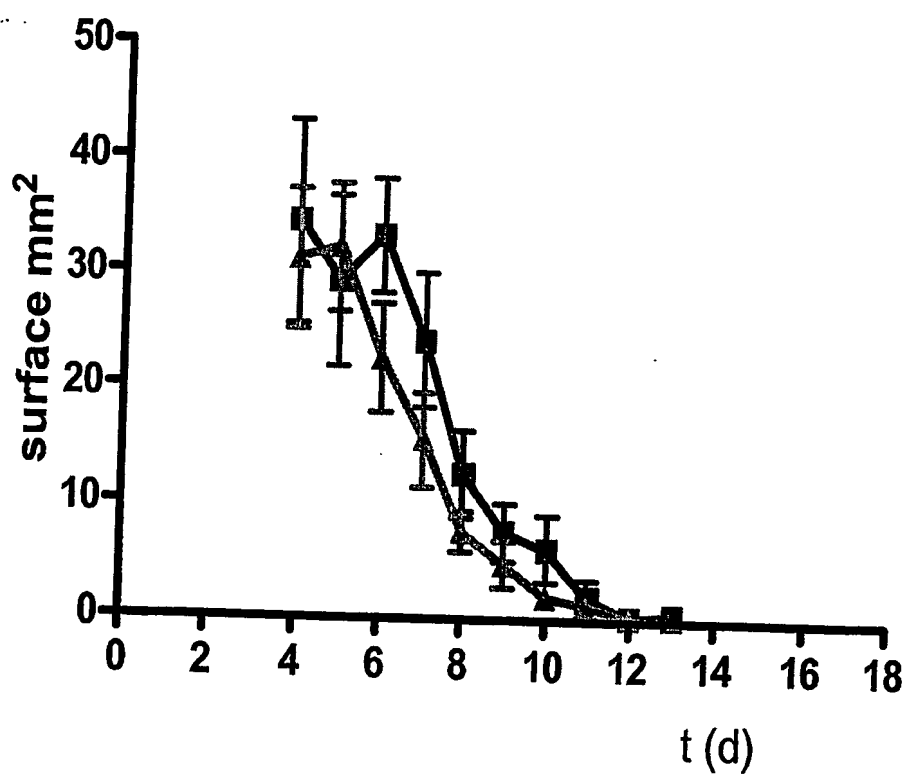
1/3

Fig. 1



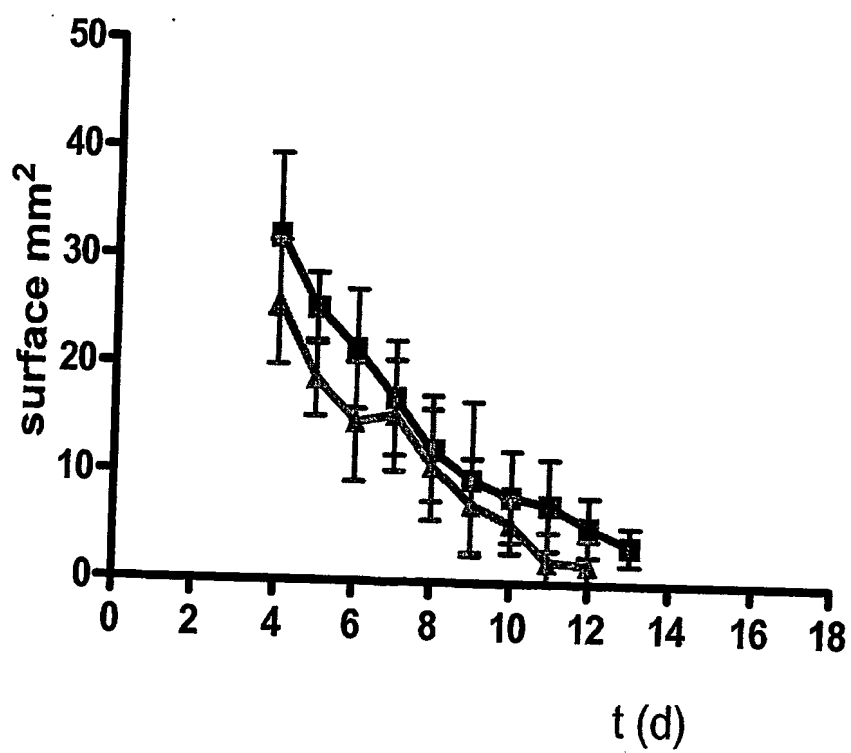
2/3

Fig. 2



3/3

Fig. 3



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/052545

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2004/002534 A (NOVO NORDISK AS) 8 January 2004 (2004-01-08) page 3 - page 5; claims 12-36	1-13
X	EP 1 072 609 A (SAKANAKA MASAHIRO ; SATO KOHJI (JP); TANAKA JUNYA (JP)) 31 January 2001 (2001-01-31) page 11, line 29 - page 15, line 32; claims 15-17; figures 8,9; examples 5-7; table 2	1-13
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

15 February 2005

Date of mailing of the international search report

01/03/2005

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/052545

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; 16 November 2001 (2001-11-16), SÁNCHEZ MARÍN B ET AL: "'Response to recombinant human erythropoietin of anemia secondary to pressure ulcers in a patient with multiple sclerosis!" XP002317776 Database accession no. NLM11785020 abstract &amp; REVISTA DE NEUROLOGIA. 2001 NOV 16-30, vol. 33, no. 10, 16 November 2001 (2001-11-16), pages 997-998, ISSN: 0210-0010</p>	1-13
P, X	<p>DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; October 2004 (2004-10), KEAST DAVID H ET AL: "Treatment of chronic skin ulcers in individuals with anemia of chronic disease using recombinant human erythropoietin (EPO): a review of four cases." XP002317777 Database accession no. NLM15509883 abstract &amp; OSTOMY/WOUND MANAGEMENT. OCT 2004, vol. 50, no. 10, October 2004 (2004-10), pages 64-70, ISSN: 0889-5899</p>	1-13
X	<p>DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; May 1992 (1992-05), TURBA R M ET AL: "Pressure sore anemia: response to erythropoietin." XP002317778 Database accession no. NLM1580782 abstract &amp; ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION. MAY 1992, vol. 73, no. 5, May 1992 (1992-05), pages 498-500, ISSN: 0003-9993</p>	1-13
A	<p>WO 99/12559 A (BRIEN JOHN S O ; UNIV CALIFORNIA (US)) 18 March 1999 (1999-03-18) the whole document</p>	1-13

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/052545

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GREGOLI P A ET AL: "The roles of Bcl-X1 and apopain in the control of erythropoiesis by erythropoietin" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 90, no. 2, 15 July 1997 (1997-07-15), pages 630-640, XP002187715 ISSN: 0006-4971 abstract</p>	1-13

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/052545

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 2004002534	A	08-01-2004	WO 2004002534 A1	08-01-2004
			US 2004038864 A1	26-02-2004
EP 1072609	A	31-01-2001	JP 2001010972 A	16-01-2001
			EP 1072609 A2	31-01-2001
WO 9912559	A	18-03-1999	AU 9774698 A	29-03-1999
			CA 2304108 A1	18-03-1999
			EP 1011709 A1	28-06-2000
			JP 2001515866 T	25-09-2001
			WO 9912559 A1	18-03-1999